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FACTOR ANALYSIS OF RELATIVE GROWTH

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INTRODUCTION

It is known that if the members of a group of organisms are measured repeatedly on the same characteristic throughout a period of time that the resulting measurements are more variable (have greater variance) at some times than at others. Attention has been called to this fact by Baker and Brooks (1947) and this fact is further well illustrated by the data on the razor clam, Baker (1943). For one group of 16 eleven-year-old clams the average lengths at the end of successive years were 0.37 (4 measurements), 2.06, 4.82, 7.48, 9.48, 11.36, 12.44, 13.22, 13.59, 14.02, 14.34 centimeters. The corresponding variances are 0.03, 0.48, 1.93, 2.07, 1.31, 0.82, 0.43, 0.22, 0.27, 0.25, 0.13, respectively. This changing variance as a group progresses through time seems to be an almost universal phenomenon and seems to result from the interplay of environmental and genetical factors.

Even though the variability of a measurement changes with time it is possible that the members of a group may occupy the same relative positions some of the time but, perhaps, not all of the time. Such a situation would lead to an examination of correlation coefficients between measurements at different dates. The complete set of possible correlation coefficients would produce a square matrix whose order is the number of separate measurements made on the group.

One of the important features of factor analysis as expounded by Cattell (1952), Holzinger and Harman (1941), and Thurstone (1947) is that a matrix of correlation coefficients may be replaced by a factor matrix with far fewer rows or columns or possibly fewer rows and fewer columns. These factors would indicate separate influences operating on the relative growth of individuals. These separate in-

fluences may be identified as environmental, physiological, or genetical effects. In any case the structure of the growth phenomenon is clearly exhibited with respect to time.

It might be thought at first glance that the analysis of variance could be used to indicate the presence and strength of a general growth factor. But such an analysis is vitiated by the above mentioned inequality of variances. Further, the rank of the correlation matrix or the number of separate factors is not determined and the separate factors are not exhibited as functions of time.

The factor analysis technique has been applied to a variety of data similar to that reported by Baker (1943), Baker and Brooks (1947), Baker and Davis (1951), and Baker, Mead, and Regan (1945). The numbers of individuals available in some cases were small but in general the method seems to be a powerful tool in the discovery and assessment of genetic, physiological, and environmental effects. The application of factor analysis to biological problems of growth seems to be novel so that one of the main purposes of this paper is to suggest such analyses.

In order to keep the paper from being unjustifiably long the somewhat detailed treatment of only one set of data, measurements on the cheek diameters of 75 Phillips cling peaches on 20 dates will be presented. For further illustration and contrast a final rotated factor matrix for 40 Nonpariel almonds for 13 dates is given.

Illustrative Data

The data on peaches consist of measurements of a linear dimension, cheek diameter, on 75 tagged Phillips clings on 20 dates that differ mainly by seven days, although some periods are shorter and some longer (see Table 1). The complete set of correlation coefficients gives rise to a 20 x 20 matrix, Table 1. The communalities, Cattell (1952), were taken as all equal to unity for the extraction of the first factor, F_1's of Table 2. Factor extraction continued as indicated by Cattell until the residuals were suitably small giving rise to the original factors F_1, F_2, F_3, and F_4 of Table 2. Actually F_4 may be an over extraction but it is better to over extract than to stop too soon.

It is seen that F_1 is quite high and uniform from May 27 to August 4 inclusive. The F_i's for these dates were averaged and k_j's determined by the method of Lagrange multipliers to make F'_1 as large as possible subject to the condition that the sum of the squares of the elements

TABLE 1

Correlations between suture diameters for certain dates for 75 tagged Phillips peaches (1942) from Sutter County, California. Date of full bloom March 15. Date of pit-hardening May 27.

		May 1	May 6	May 12	May 16	May 20	May 27	June 2	June 9	June 16	June 23	June 30	July 7	July 14	July 22	July 29	Aug. 4	Aug. 12	Aug. 22	Sept. 2	Sept. 8
May	1	1.00	.95	.82	.70	.53	.36	.28	.26	.27	.27	.29	.28	.32	.35	.60	.36	.30	.24	.05	.002
	6		1.00	.93	.84	.69	.53	.47	.44	.45	.45	.63	.46	.50	.53	.54	.53	.66	.31	.41	.33
	12			1.00	.97	.88	.77	.74	.71	.72	.71	.73	.72	.75	.75	.75	.74	.68	.53	.39	.35
	16				1.00	.95	.87	.84	.81	.82	.68	.83	.82	.85	.84	.84	.83	.76	.62	.48	.44
	20					1.00	.94	.93	.91	.91	.90	.91	.89	.91	.89	.88	.92	.82	.69	.55	.52
	27						1.00	.97	.96	.95	.94	.94	.93	.93	.90	.89	.91	.83	.74	.62	.60
June	2							1.00	.98	.99	.98	.97	.73	.96	.93	.91	.90	.90	.79	.68	.63
	9								1.00	.98	.98	.97	.96	.96	.93	.91	.89	.85	.78	.67	.63
	16									1.00	.99	.99	.98	.93	.94	.92	.90	.88	.79	.67	.65
	23										1.00	.99	.98	.97	.95	.93	.91	.89	.81	.70	.66
	30											1.00	.99	.98	.96	.94	.93	.90	.82	.71	.68
July	7												1.00	.98	.96	.94	.93	.90	.82	.71	.67
	14													1.00	.98	.96	.96	.91	.81	.70	.67
	22														1.00	.98	.98	.93	.82	.72	.67
	29															1.00	.99	.94	.91	.71	.67
Aug.	4																1.00	.95	.83	.74	.67
	12																	1.00	.92	.82	.77
	22																		1.00	.96	.93
Sept.	2																			1.00	.98
	8																				1.00

TABLE 2

Extracted factors of growth for 75 Phillips cling peaches rotated by an orthogonal λ-matrix giving maximum definition of the rotated factors.

Dates		Original Factors				Orthogonal λ-matrix				Rotated Factors			
		F_1	F_2	F_3	F_4	F'_1	F'_2	F'_3	F'_4	F'_1	F'_2	F'_3	F'_4
May	1	.468	—.827	—.211	.312	.982	—.168	—.092	.000	.330	—.917	.127	.270
	6	.662	—.740	—.282	—.296	.127	.927	—.339	.098	.511	—.893	—.140	—.293
	12	.832	—.564	.081	—.040	.142	.338	.901	—.233	.756	—.635	.177	—.112
	16	.897	—.408	.121	.109	.018	.000	.257	.968	.848	—.488	.193	.037
	20	.945	—.182	.220	.010					.936	—.253	.175	—.059
	27	.942	.033	.213	.036					.960	—.056	.103	—.012
June	2	.942	.050	.209	—.229					.957	—.041	.026	—.265
	9	.942	.182	.246	—.034					.982	.094	.065	—.072
	16	.951	.180	.228	—.065					.988	.084	.040	—.098
	23	.949	.239	.180	—.100					.986	.123	—.032	—.115
	30	.975	.126	.132	—.125					.990	—.002	—.046	—.139
July	7	.946	.170	.155	.157					.975	.051	.035	.133
	14	.968	.132	.150	.056					.990	.010	.016	.032
	22	.967	.116	.080	.063					.977	—.028	—.040	.054
	29	.978	.046	—.092	.305					.959	—.153	—.110	.321
Aug.	4	.959	.093	.022	.132					.959	—.066	—.066	.132
	12	.944	.138	—.188	—.054					.917	—.094	—.289	.005
	22	.859	.341	—.365	.089					.837	.048	—.501	.205
Sept.	2	.754	.375	—.474	—.160					.718	.060	—.665	—.008
	8	.712	.391	—.455	—.153					.681	.089	—.647	—.004

of the column is one. These k_j's are entered as the first column, F'_1, in the λ-matrix of Table 2. The second column of the λ-matrix is determined to make F'_2 as large as possible at the first two early dates. Now two conditions are imposed in the Lagrange method by one orthogonality condition of the second column with the first column and by the condition that the sum of squares of the elements of the second column is one. The third column of the λ-matrix was determined to make F'_3 as large as possible at the last two later dates. The maximizing must be carried out subject to two orthogonality conditions and one condition on the sum of squares. The last column of the λ-matrix is then fixed. As far as is known these methods of rotating to simple structure are not in the textbooks and are probably possible only because of the regular development and fading of the three factors of growth. Multiplication of the F_t-matrix by the λ-matrix gives the F_t'-matrix.

The factor F'_4 is seen to be very small and unimportant so that by means of factor analysis we have reduced a 20 x 20 correlation matrix to a 3 x 20 factor matrix.

An inspection of the F'_t's of Table 2 indicates rather clearly that three factors are necessary to explain the relative growth of these 75 peaches. There is a central time of stability in the relative positions of the peaches extending from May 27, the date of pit hardening, Baker and Davis (1951), until August 4. Sizeable loadings on this factor occur at all dates. The second factor is most clearly associated with early growth and since the loadings are negative, it is indicated that peaches relatively large at one early measurement tend to be relatively small at a near later measurement. This could be due to competition between fruits and early dropping of losers in the competition with consequent rapid growth of the survivors. Factor 2, F'_2, complements factor 1 in the early stages of growth and fades away as factor 1 increases. Factor 3 complements factor 1 in the late stages of growth much the same as factor 1 does in the early stages. Factor 3 is again negative which probably indicates that the early ripening fruits complete their growth earlier than some which ripen later which tends to reverse the relative positions of the fruits as they pass through time.

One difficulty with factor analysis is that the size of a loading required for significance is not very exactly known. However, in the present case the general picture seems quite clear. There is some

doubt as to whether the loadings on factor 3 are significant at the earlier dates.

For purposes of further illustration and comparison the rotated factor matrix for 40 Nonpariel almonds for 13 dates is given in Table 3. The matrix in this case is extremely similar to the corresponding

TABLE 3
Rotated factor matrix for 40 Nonpariel almonds for 13 dates.

Dates		F'_1	F'_2	F'_3	F'_4
March	13	.271	—.928	—.050	—.108
	20	.440	—.897	.047	—.001
	27	.509	—.841	.137	.013
April	4	.695	—.669	.253	—.032
	13	.940	—.222	.253	—.057
	20	.970	—.089	.214	.041
	27	.996	—.003	.108	.038
May	4	1.003	.017	.004	.040
	11	1.003	.020	—.021	.016
	21	.998	.029	—.007	—.076
	28	.997	.009	—.039	—.064
June	4	.998	—.030	—.015	—.051
	11	.996	—.036	—.030	—.050

part of Table 2 except that the factor 3 loadings are zero for the later dates. This corresponds to our knowledge of the difference in growth of almonds and peaches for the almonds do not have the final swell in size that is characteristic of the growth of peaches.

Summary

If repeated measurements at different dates are made on the members of a group the correlation coefficients between the measurements for the different dates can be calculated. These correlation coefficients form a n x n matrix where n is the number of dates. By the methods of factor analysis the n x n correlation matrix can be reduced to a p x n factor matrix where p is usually much less than n. Rotation to simple structure can be accomplished by the method of Lagrange's multipliers where the conditions are suitably chosen. As an end result the number of factors is exhibited and their interplay with each other as time progresses is indicated.

Some illustrative data are treated in some detail for material whose growth characteristics have been studied extensively. The factor analysis gives very reasonable results in these cases. This forms a basis for the hope that the methods indicated here may be helpful

in preliminary stages of the study of growth under other conditions and with other materials. Other materials have been considered on a rather limited basis as far as numbers of individuals are concerned. The methods of factor analysis seem to be very helpful in the identification and assessment of the importance of various genetical, physiological, and environmental factors on growth.

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COMPARISON OF THE AMINO ACID COMPOSITION OF ALCOHOLIC EXTRACTS OF NEOPLASTIC TISSUES WITH SIMILAR EXTRACTS OF NORMAL MUSCLE[1, 2]

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Alcoholic extracts of rat sarcomas were analyzed in an earlier study (28), in order to determine their main components and how these might differ quantitatively or qualitatively from the components of extracts made in a similar way from normal rat tissue. The reproducible results showed several significant differences between the tumor extracts and all the extracts of the normal tissues. There were, for example, marked differences in the ash, inorganic phosphorus, glucose, lactic acid, desoxyribonucleic (DNA) and ribonucleic (RNA) acids. In that study the nitrogenous constituents were not examined in detail, except the RNA and DNA, which together accounted for 15 to 36 per cent of the total nitrogen. The remaining nitrogen, chiefly amino acid, peptide or protein nitrogen, is the subject of this study.

The possible significance of the nature of the protein in tumors has been a stimulus for extensive work in this field, which was reviewed by Toennies in 1947, (34). Since then much work has been done on the physical chemistry of the tumor proteins extracted with salt solutions (16) (21) (29) (30) (31). These studies showed that the tumor proteins were large molecules which are different from the proteins similarly obtained from normal tissue. Such proteins are probably not concerned to any extent in the present study, since only substances soluble in 80 per cent alcohol would be included in these extracts.

Early in the work it became obvious that mainly free amino acids were present in the extracts or only very small protein or polypeptide

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[2] Presented before the American Society of Biological Chemistry, Atlantic City, April 13, 1954.

molecules. The question as to whether there may be a small amount of anything more complex than the single amino acids will be carefully considered, especially in view of the oncolytic and immunizing capacity of these extracts (1) (2) (14) (28). Whether there are present in the tumor extracts different amino acids or only different proportions of the same amino acids from those found in the extracts of normal tissues will also be discussed.

Materials and Methods

Source of extracts—

The extracts used in these experiments were the same as those analyzed in the previous study (28). They consisted of alcoholic extracts of homogenates made from rat sarcomas (T); from the skeletal muscle tissues from normal rats (N); from skeletal muscle of rats bearing the sarcomas (NT); and from rats from which the treated tumors had been sloughed and which then had been shown to be resistant to further challenge (NIm). The rats were generously supplied to us by Dr. Margaret Reed Lewis and Dr. Paul Aptekman of the Wistar Institute and were all from strictly inbred strains. The extracts from the Lewis rats are prefixed with an L. The extract L-T was from sarcoma No. 10, LT-II from sarcoma No. 7, and LT-III was from a rat lymphoma No. 8. The extracts T-I and T-II were from sarcomas No. 6.

Method of preparation of the extracts—

The method of preparation was identical for all the extracts and is given in detail in the previous paper (28). It will be briefly recalled that the original tissue was homogenized in a Waring blendor with a volume of 95 per cent alcohol equal to the weight of the tissue. After 24 hours at 3 to 4°C the supernatant was made to a final concentration of 80 per cent alcohol by the addition of two volumes of 95% alcohol. After several more days the precipitate was removed. The filtrate was concentrated in vacuo at 30°C giving an alcohol concentration of 30 per cent. The final solution was clarified by filtration at —15°C. The amount of solids per milliliter in these extracts was determined, and calculations of all components were made on a solid weight basis.

Aliquots of the concentrated extracts were dried in vacuo to remove the alcohol and then hydrolyzed with 200 times their weight of six

normal hydrochloric acid for 18 hours at 102-103°C, as recommended by Moore and Stein (17). The acid was then removed under vacuum. The solution was made to volume with tenth normal hydrochloric acid and centrifuged free of humin.

Nitrogen analyses—

Total nitrogen was measured by the micro method of Markham (15). Free ammonia was determined by adding sodium carbonate directly to the extract, aerating into saturated boric acid and titrating by a procedure similar to that used for the total nitrogen. Urea nitrogen was determined in the same way on extracts treated with urease for 15 minutes previous to aeration. Analyses for free amino nitrogen were made by the manometric ninhydrin method of Van Slyke, Dillon, MacFadyen and Hamilton (35).

Starch column chromatography—

Starch columns (0.9 x 30 cm.) were prepared exactly as described by Stein and Moore (33), and 0.3 to 0.6 ml. amounts of extracts or their hydrolysates were applied to the columns in 1:2:1 (n-butyl-n-propyl-0.1 N-HCl) solvent. Elution was performed with this solvent, followed by 2:1 (n-propyl-0.5 N-HCl) solvent, and aliquots were collected by means of the drop-counting device described (33). Usually two experiments were made simultaneously, a second one being run in tandem with the one using the drop counter. Measurements of the effluents were made by means of the ninhydrin method of Moore and Stein (17). From these values it was possible to calculate the effluent recovery from the starch columns.

Experiments were carried out on both unhydrolyzed and hydrolyzed extracts, which were diluted to the proper concentrations with 1:2:1 solvent and applied directly to the columns.

Paper chromatography—

Ascending paper chromatograms were made on Whatman No. 1 paper 8 x 8 inches. These were formed into cylinders and stood in closed bottles in a layer of phenol reagent,[3] prepared according to Berry (6). The second solvent was butanol-acetic solvent[4] (6).

[3] The phenol reagent according to Berry consisted of 100 g. phenol saturated with an aqueous solution containing 6.3% sodium citrate and 3.7% NaH_2PO_4 or KH_2PO_4.

[4] Butanol-acetic acid solvent according to Berry was 80 ml. n-butanol, 20 ml. glacial acetic, and 20 ml. water made fresh for each determination.

When the solvents reached nearly to the top of the paper the latter were withdrawn, dried 5 minutes in air and then in a hot air oven at 90°C. After they had been run consecutively at right angles in the two solvents, they were sprayed with a 0.2% solution of ninhydrin in n-butanol saturated with water and again dried for 4 minutes at 90°C.

Bioautography with mutants[5] *of Escherichia coli*—

This technic was suggested by Davis (7) as highly sensitive and specific for the detection of certain amino acids. The mutant strain of coli No. 173-25, which requires diaminopimelic acid for growth, was used to detect the presence of this amino acid. Plates of solid basic medium (8), supplemented with lysine, were seeded with the coli organisms, and then the excised area of a paper chromatogram containing the suspected amino acid was laid in contact with the surface of the plate. In twenty-four hours colony growth appeared only under the site of the specific amino acid.

The mutant coli strains No. 39A-32, 32-4, and 57-29, which were dependent upon cystine, were also seeded in plates of the simple basic medium and used for detecting the presence of cystine in the same manner as described above.

Paper electrophoresis—

Paper electrophoresis experiments were carried out on both the alcoholic extracts and pure amino acids. About 0.004 ml. of solution was applied in a line on 2 inch strips of Whatman No. 1 filter paper and run in the paper strip model electrophoresis apparatus obtained from Arthur H. Thomas Co., using 130 volts and 25 milliamperes for 2 hours in barbiturate buffer, pH 8.6, made as follows: sodium barbiturate (29.428 gm.), sodium acetate 3 H_2O (19.428 gm.), and N/10 HCl (180 ml.) were dissolved in 3000 ml. distilled water and then diluted with an equal amount of distilled water. At the end of the experiment the strips were dried for 4 minutes on the rack in an oven at 90°C, sprayed with the 0.2% solution of ninhydrin in n-butanol saturated with water and again dried for 4 minutes at 90°C.

Quantitative evaluation of the color bands was made from diagrams drawn by means of a densitometer.[6] The areas under the peaks were

[5] Cultures of these mutant strains were kindly given to us by Dr. B. D. Davis.
[6] This densitometer was designed by Arthur H. Thomas and kindly loaned to us.

measured with a planimeter, and from the total areas were calculated the percentages of the total concentration as each component.

Results

Total nitrogen—

The total nitrogen of the extracts included nitrogen of the nucleic acids, amino acids or proteins, free ammonia, amide nitrogen, urea nitrogen, and any other unidentified nitrogen present. Since it was shown (28) previously that the ash content of the tumor extracts (10.4 to 20 per cent of the total solids) was lower in general than that of the normal tissue extracts (20.2 to 35.9 per cent), all calculations for this study were made on the basis of ash-free total solids. The values for the nitrogen due to DNA and RNA were taken from analyses found previously on these same extracts and recorded in an earlier paper (28). "Net nitrogen" was found by subtracting from the total nitrogen that nitrogen due to the DNA, RNA, ammonia and urea and in all the extracts it varied from 4.6 to 8.4 per cent of the total ash-free solids.

Free ammonia—

The amount of free ammonia nitrogen represented very little (0.3-0.5 per cent) of the total nitrogen of the normal tissue extracts and even less (0.02 to 0.08 per cent) of the tumor extracts.

Urea nitrogen—

The urea nitrogen content of the normal and tumor extracts varied between 0.4 to 2.0 per cent of the total ash-free solid weight and did not appear to differ significantly between the normal and tumor tissue extracts.

Amino nitrogen—

The free amino nitrogen was considerably more (1.8 to 3.5 per cent) in the tumor extracts than in the normal tissue extracts (1.1 to 1.7 per cent of the solid ash-free weight). The difference was very striking when calculated as percentage of the total net nitrogen (56.0-59.2 per cent for tumor tissue as against 17.8-22.9 per cent for normal tissue extracts). These results indicate that a large amount of the extracts existed as free amino acids, especially in the tumor extracts, and this was confirmed by the chromatography experiments (see below).

However, when representative solutions (two normal tissues and

two tumor extracts) were hydrolyzed, the free amino nitrogen contents in both types of extracts increased, indicating that some peptides or whole proteins were originally present in the extracts. The actual percentage of this increase in free amino nitrogen was 12 to 17 per cent in the case of the tumor extracts and considerably more in the normal tissue extracts (56 to 93 per cent).

Starch column chromatography analyses—

When the extracts were placed directly on starch columns and analyzed by the technic described by Moore and Stein (18), the effluent patterns (Figure 1 and 3) obtained for the extracts from the skeletal muscle tissue from normal rats, from rats bearing tumors, and from resistant rats were essentially similar, but all of these were different from the ones obtained with extracts from the tumors (Figure 2 and 4). The constancy with which the main peaks appeared shows that the method of preparing the extracts affords reasonably reproducible products. It is evident that a large proportion of the extracts existed as free amino acids. The total ninhydrin-reacting substances evolving from the columns, when compared with the content of the original solutions used on the columns, showed 90-100 per cent recovery. The percentage of the total amino acids found in this way for only the main components are assembled in Table 1 under the effluent values at which they appeared, and the pure amino acids found to appear at these positions under the same conditions are given at the heads of the columns. Small amounts, no more than two to three per cent, of several other minor components were also found, such as those corresponding with effluent values of aspartic acid, ammonia, glucosamine and lysine, but these are not included in the Table. The diagrams of the unhydrolyzed solutions showed overlapping of some amino acids and perhaps some interference with their evolution, as evidenced by the heterogeneous peaks.

Four of the solutions (two normal tissue extracts and two tumor extracts) were then hydrolyzed, and their analyses, made in the same way, were recorded in Table 1 in parenthesis to the right of the analyses of the corresponding unhydrolyzed solution values. The peaks for these hydrolyzed solutions were much sharper (Figures 3 and 4) than those of the unhydrolyzed solutions (Figures 1 and 2) and permitted better identification with known amino acids.

The main differences between the normal and tumor tissue extracts

TABLE 1

Comparison of Amino Acid Content of Normal and Neoplastic Rat Tissue in Terms of Percent of Total Concentrations at Different Effluent Values

Range Found for Effluent Values in ml.	4.4	11.4-15.3	15-27	48-52	55-65	67-77	89-94	97-104	106-114	123-144
Effluent Values of Known Amino Acids in ml.	?	Leucine (13.5)	Phenylalanine (16) Tryptophane (17) Valine (24) Tyrosine + Methionine (26)	Proline (51)	Glutamic + Alanine (58) Cysteic Acid (65)	Taurine (74) Threonine (75)	?	Serine (100) Ethanolamine (103)	Glycine (108)	Arginine (136)
Extracts	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Muscle NN-I	0 (0)	0 (1.0)	3.6(0.9)	1.3(0.8)	11.7(11.3)	40.3(15.5)	1.8(7.7)	17.3(11.2)	10.1(32.6)	8.3(13.0)
NN-II	0 (0)	0 (1.7)	3.6(2.4)	0.3(0.8)	13.7(10.4)	33.1(21.2)	1.2(6.2)	19.3(11.7)	16.6(28.0)	7.6(11.6)
Muscle NT-I	0	1.2	4.0	0	14.0	37.2	2.8	7.5	14.1	13.2
NT-II	1.2	0	5.2	2.0	17.8	26.9	3.6	14.5	16.6	5.9
Muscle NImI	1.2	0	3.3	0	11.3	32.0	1.5	14.8	21.9	6.0
Tumor T-I	1.5(0)	1.1(4.3)	7.8(3.5)	2.2(2.6)	33.6(22.5)	23.3(15.3)	1.8(0)	19.3(17.5)	1.3(24.2)	3.4(2.9)
T-II	1.1(0)	0.9(5.0)	7.8(3.2)	8.8(3.9)	30.1(25.6)	23.7(14.8)	3.0(0.8)	15.4(15.6)	3.2(20.8)	1.0(2.4)
LT-I	0.7	0	5.0	2.5	28.0	31.3	1.6	20.8	1.7	1.7

Data in parentheses to the right of the percentage values represent the results on the hydrolyzed extracts.

Figures in parentheses following the names of the amino acids represent the theoretical effluent values of Moore and Stein obtained on a synthetic mixture.

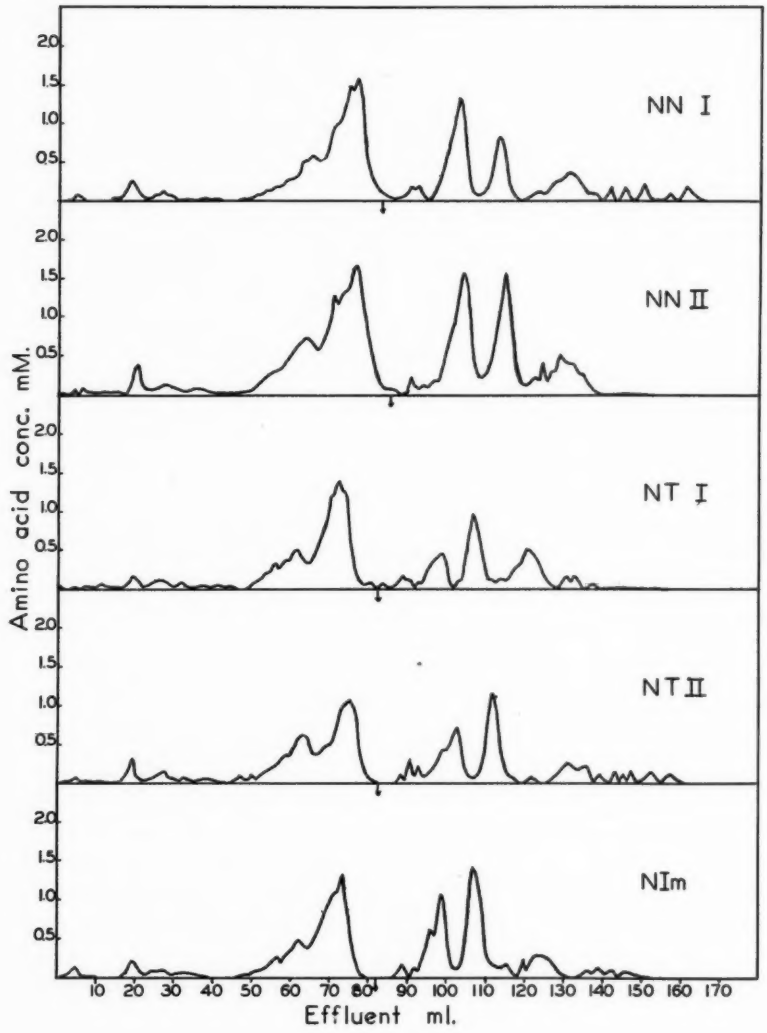

FIGURE 1

Chromatographic analyses of unhydrolyzed extracts of muscle tissue from normal (NN-I, NN-II), tumor-bearing (NT-I, NT-II), and resistant (N-Im) rats.

may be seen from further examination of Table 1 and Figure 5. There was in all tumor extracts considerably more of the component corresponding to glutamic acid + alanine than in any of the normal tissue extracts, and while it decreased somewhat after hydrolysis of the extracts still it was greater. More proline, as well as the group of neutral amino acids, phenylalanine, valine, tyrosine, methionine, and tryptophane, was present in the tumor extracts than in the normal tissue extracts, although the concentration of these components was relatively small in all cases. There was in the tumor extracts also a small peak at an effluent value of 4.4 ml. which disappeared on hydrolysis. This peak was completely absent in the normal muscle

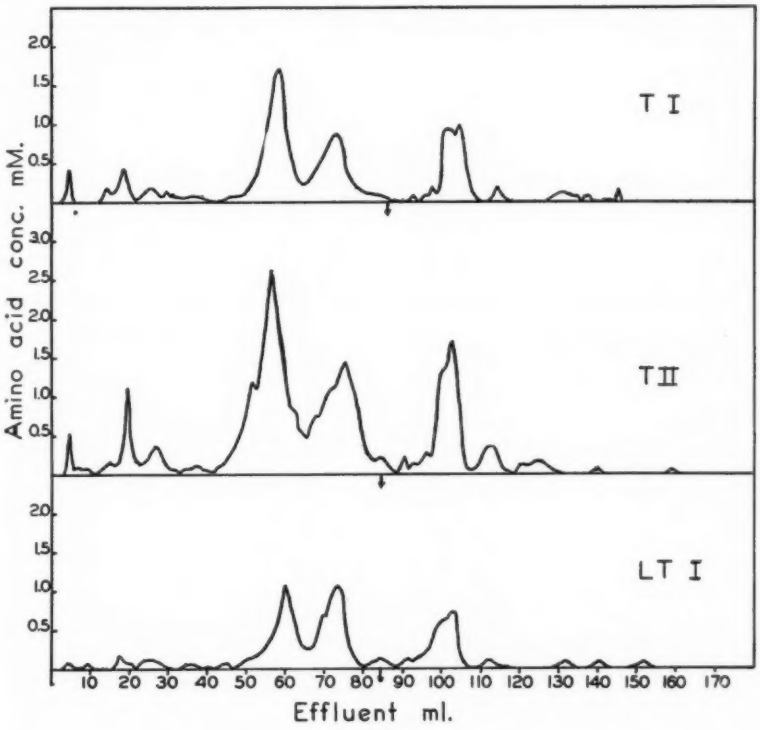

FIGURE 2
Chromatographic analyses of unhydrolyzed tumor (sarcoma) extracts (T-I, T-II, LT-I).

from normal rats, but was present to some extent in the normal muscle from tumor-bearing or immune rats. It could be a component of carbohydrate and ammonia. On hydrolysis, a small amount of something having the same effluent value as leucine also appeared in greater amount in the tumor than in the normal tissue extracts.

On the other hand, the normal tissue extracts showed somewhat more of the component with the effluent value of taurine or threonine than did the tumor extracts, and on hydrolysis the percentage of this component in both types of extracts decreased and the peak sharpened

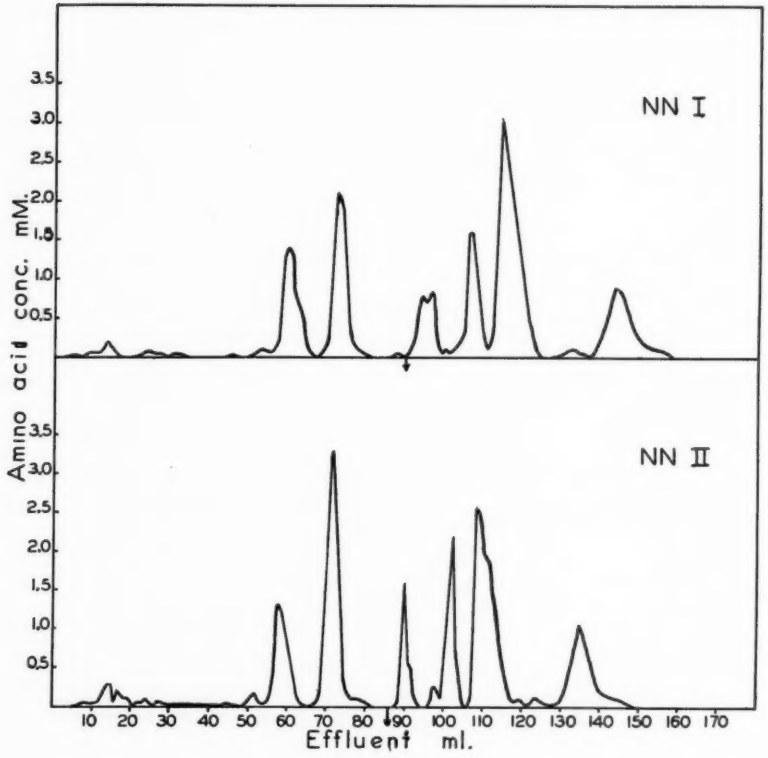

FIGURE 3
Chromatographic analyses of hydrolyzed extracts of muscle tissue from normal (NN-I, NN-II) rats.

and suggested its identification with taurine. In the normal tissue extracts also more arginine was present than in the tumor extracts, and it increased still more on hydrolysis. Much more of the component corresponding to glycine was present in the unhydrolyzed normal extracts than in tumor extracts. On hydrolysis it increased greatly in both types of extracts, especially in the tumor extracts, until finally its concentration was only slightly less than that in the normal tissue extracts.

An unidentified component appeared in very small amount in all unhydrolyzed extracts at an effluent value of 90 to 91 ml. It increased

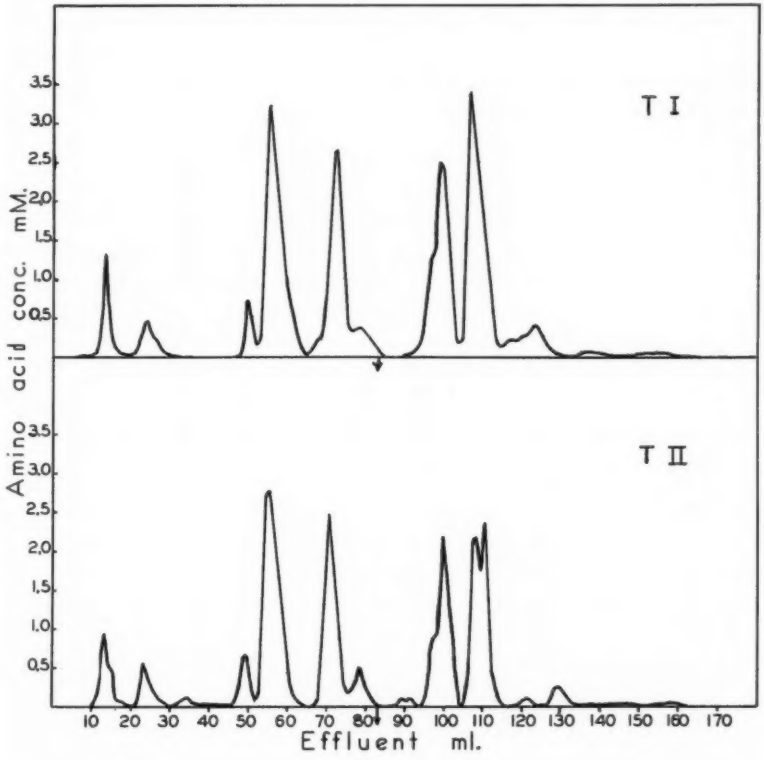

FIGURE 4
Chromatographic analyses of hydrolyzed extracts of tumor (sarcoma) (T-I, T-II).

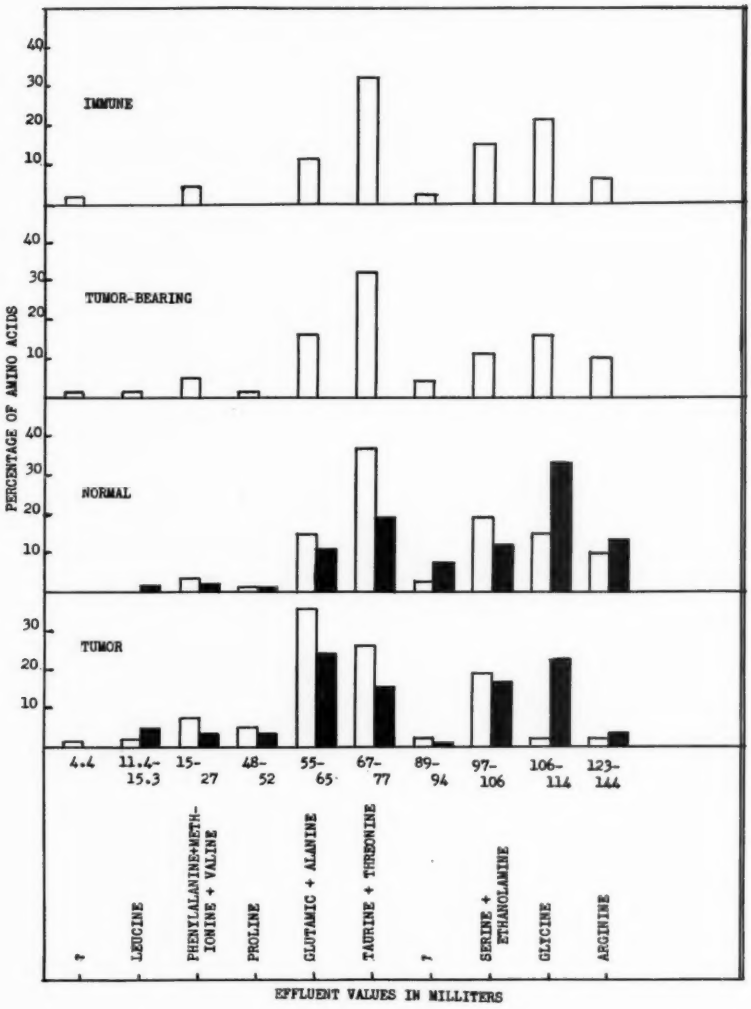

FIGURE 5

Comparison of the concentration of the amino acids in the unhydrolyzed (open blocks) and in the hydrolyzed (black blocks) extracts.

noticeably following hydrolysis only in the normal tissue extracts. It has been suggested by Dr. S. Moore that this might be β-alanine, and that the increase in the peak at the location of arginine might be due at least in part to 1-methyl-histidine, these two substances being hydrolysis products of anserine, which occurs in muscle.

Paper chromatographic analyses—

Samples of normal tissue extract and of tumor extract were studied by two dimensional chromatography, using phenol and then butanol acetic acid solvents. The results obtained by this method confirm those with the starch column chromatography and free amino nitrogen analyses, by indicating that the unhydrolyzed extracts were rich in free amino acids, and by showing that there were striking differences between the normal and tumor tissue patterns.

The chief differences in these unhydrolyzed extracts were as follows. Only the tumor extracts contained aspartic acid, ethanolamine and small amounts of a number of other amino acids, such as cystine, valine, phenylalanine, proline and ethanolamine phosphoric ester. Their content of glutamic acid was higher. On the other hand their content of taurine, arginine and lysine was lower than in the normal tissue extracts. Both contained considerable alanine and glycine or serine and some leucine and threonine.

After the extracts were hydrolyzed some new amino acids spots appeared, and the amounts of others were increased or in some cases decreased. Again the patterns for the tumor extracts were readily distinguishable from those of the normal extracts. The most easily noted differences were the presence of a blue spot exclusively in the normal tissue extract in the position of histidine or 1-methyl histidine,[7] and the presence exclusively in the tumor tissue of proline and of a group of definite spots with low R_F values in both phenol and butanol-acetic acid solvents. In this group were spots with R_F values corresponding to those of aspartic acid, cystine, lanthionine,[7] and α-ϵ-diaminopimelic acid.[7] Some of these spots appeared also in traces occasionally in the normal tissue extracts.

Both normal and tumor tissue extracts showed considerable glu-

[7] We are grateful to Dr. Elizabeth Work and Dr. Lemuel D. Wright for samples of diaminopimelic acid, to Dr. J. Awapara for ethanolamine phosphoric ester, to Dr. T. F. Lavine and to the California Research Foundation for samples of cystathionine, and to the latter for a number of the other rare amino acids, viz., lanthionine, cysteic acid, homocystine, 1-methyl-histidiene, carnosine and sarcosine.

tamic acid and alanine, some ethanolamine phosphoric ester,[7] serine or glycine, threonine, valine and leucine. Spots corresponding to glutathione, taurine, tyrosine, methionine and phenylalanine also occasionally appeared.

Since the presence in tumor extracts of an amino acid such as α-ε-diaminopimelic acid (DAP), which has so far been found (3) (37) (38) (39) (40) only in certain bacteria and algae, would be highly significant, further study was made to identify the spots in the area corresponding to an R_F of less than 0.20 in phenol and less than 0.10 in butanol acetic acid. The known amino acids appearing in this area under the conditions of the experiment were cysteic acid, cystathionine,[7] lanthionine, cystine, and diaminopimelic acid (see Table 2). All of the seven hydrolyzed tumor extracts showed two spots, one with R_F values within the limits for cystine, and one with R_F values within the limits for α-ε-diaminopimelic acid or lanthionine, which have overlapping R_F values. No spots appeared with values low enough to correspond with cysteic acid[7] or with the low value cystathionine spot.

TABLE 2
R_F Values

	Phenol	Butanol-Acetic
Cysteic acid	0.04	0.05
Cystathionine	0.071 0.154	0.019 0.045
Lanthionine	0.13	0.04
Cystine	0.17–0.20	0.05–0.08
α-ε-diaminopimelic acid	0.12–0.13	0.03–0.06
Hydrolyzed Seven tumor extracts	0.09–0.13 0.15–0.23	0.02–0.06 0.06–0.09

The conclusion based on paper chromatographic diagrams must, therefore, be that in addition to cystine either α-ε-diaminopimelic acid or lanthionine existed in hydrolyzed extracts of the tumors.

Identification of amino acids by means of Escherichia coli mutants.

The question as to whether the spot under consideration might be α-ε-diaminopimelic acid was pursued by means of the highly specific and sensitive test suggested by Davis (7) described under methods. When the undeveloped paper chromatograms of the hydrolysates which

[7] See footnote, page 157.

had been run in the two solvents were placed on large plates seeded with the 173-25 coli mutant, no halo of colonies appeared, whereas such a halo did appear under a spot of known diaminopimelic acid. Since the concentration of the unknown substance in the spot from the hydrolysate may have been too low to afford obvious growth, drops of the extracts were directly applied to the plate. When this was done definite growth did appear on repeated tests, indicating the presence of diaminopimelic acid in the tumor extracts. When the test was made in the latter way, positive growth was found for the hydrolyzates of T-I, T-II, LT-I (faint), LT-II, LT-III, NN-I, NN-II, LN-I, LN-II, and LImI, as well as for pure α-ε-diaminopimelic acid. Negative tests were found for cystine, cystathionine, homocystine, and the unhydrolyzed extracts of NN-II, LT-I, LT-II, and LT-III. Faint reactions were found for the unhydrolyzed extracts of NN-I, T-I and T-II. However, at a later date, when these tests were repeated, no growth appeared under any of the drops of the hydrolysates tested, while α-ε-diaminopimelic acid did induce growth. At this time cystathionine was also found to induce growth but the colonies appeared to be coarser and fewer than with α-ε-diaminopimelic acid. Cystine and homocystine gave negative results. Similar results were again obtained and samples of cystathionine from two different sources[7] also gave positive results. We are at a loss to explain the discrepant results on the two occasions with both the hydrolyzed extracts as well as with cystathionine, except possibly on a mutation basis, since in both cases the controls were adequate, and the same results were obtained repeatedly. Lanthionine was then tested because of its resemblance to α-ε-diaminopimelic acid in structure, and it also was found to induce growth of this coli mutant (173-25). Obviously further work must be done to identify the substance responsible for the spot which has the R_F value of α-ε-diaminopimelic acid or lanthionine, and which appears only after hydrolysis and chiefly in the extracts of the tumor tissues.

Similar technic was employed, using three other mutants of *Escherichia coli* which were dependent upon cystine, and the presence of this amino acid was detected in the T-I and T-II hydrolysates. In the case of the T-I hydrolysate two spots appeared in close proximity, indicating the presence of two sulfur amino acids.

Electrophoresis

Several attempts were made to study these extracts in the Tiselius electrophoresis apparatus, but without success, because most of the material dialyzed during the preliminary equilibration of the solutions.

Therefore, a preliminary survey of the bands appearing on paper electrophoresis was made. One large group of substances, representing practically 75 per cent of the normal tissue extracts, migrated out to an intermediate position corresponding to the mobility of a large group of neutral amino acids, each examined independently under the same conditions. Minor amounts of the extracts had a higher mobility, corresponding with the basic amino acids arginine and lysine. Thirty-five per cent of the tumor extract, but only 5 to 13 per cent of the other extracts, appeared to have almost no mobility, similar to the acidic amino acids, aspartic or glutamic acids. The fact that relatively little aspartic acid was found by both the starch column and paper chromatographic technic is additional confirmation of a high content of glutamic acid in the tumor extracts. Further work is in progress.

Discussion

The free amino acid patterns here obtained by the starch column chromatographic technic, which were in general confirmed by paper chromatograms on unhydrolyzed alcoholic extracts of malignant tissue, differed obviously from those for the extracts of the host non-tumorous skeletal muscle-tissue, whether from tumor-bearing rats, rats made resistant to implantation of the tumor, or normal rats. The most conspicuous differences between the normal and the neoplastic tissues were in the relative quantities of two of the amino acids, glutamic acid and arginine. The former predominated in the tumor extracts and the latter in the normal tissues. However, it is possible that the most significant differences between the two types of tissues lay in some of the components occurring in less amount.

Kögl and Erxleben (13) had found not only more total glutamic acid, but also more of the dextro form of this amino acid in malignant than in normal tissues. Most investigators, however, have differed on the latter point (34). The type of glutamic acid present was not studied in our experiments. Characteristically different amino acid patterns for neoplastic and normal tissue extracts were reported by Roberts and his coworkers (22) (23) (24) and by Kit and Awapara

(12), who also studied alcoholic extracts and used only the paper chromatographic technic. Differences found by other workers, especially in other than alcoholic extracts, were reviewed by Toennies (34), who pointed out that many of the earlier investigators suggested a larger percentage of highly soluble, "uncoagulated" protein, or nitrogenous water extractable fraction in malignant than in normal tissue. On the other hand, Sauberlich and Baumann (25) found no significant differences in the amino acid content of normal and neoplastic rat tissues, except in a mammary fibro-sarcoma, where there was a high amount of glycine and proline. Recent studies on the amino acids of tumors have been reviewed by Griffin (11).

In the present studies the increase in free amino nitrogen, as well as the changes in the chromatographic patterns of the amino acids following hydrolysis of the extracts, indicate that some of the nitrogenous material existed in the form of peptides or whole protein. More was present in the normal tissue than in the tumor extracts. The starch column chromatographic patterns of the unhydrolyzed extracts, when contrasted with those from the same extracts after hydrolysis, seem to indicate a certain degree of inhibition in the former to elution from the columns. This appeared to be the case especially in the regions of the glutamic acid, the taurine, and in some cases the arginine, and may be due to the presence of peptides. After hydrolysis the amino acids came clean from the columns. (See Figures 2 and 4).

Hydrolysis of the extracts of normal tissue gave rise to an increase in glycine, arginine and histidine or 1-methyl histidine, amino acids all known to occur normally in the body. It suggests that they exist in complex or peptide form in the original extract. Schurr, et al. (26) noted an increase in histidine in hydrolysates of normal muscle and indicated that it probably originated from carnosine, which occurs in large amounts in muscle (36). Suggestive evidence for the presence of anserine has been mentioned earlier in this paper.

Hydrolysis of the tumor extracts did not give rise to histidine, but rather to a group of different amino acids, among which were cystine and α-ϵ-diaminopimelic acid or lanthionine.

Although Roberts and Frankel (23) reported an unknown constituent called by them an "Under Glutamic" spot in their chromatograms in the neighborhood of the position for these spots, it probably was not the same as found by us, since they reported its disappearance following hydrolysis and believed it to be a peptide, while ours ap-

peared mainly after hydrolysis. Awapara, Landua and Fuerst (5) also gave data on the "Under Glutamic" component in their unhydrolyzed extracts, and later Kit and Awapara (12) apparently designated[8] this substance as ethanolamine-phosphoric ester. However, they found even larger quantities of it in normal lymphoid tissue than in the lymphosarcomas, which was contrary to the claims of Outhouse (19) (20) that ethanolamine phosphoric ester occurred specifically in malignant tissue. We have found this amino acid in both the normal and tumor tissue hydrolysates.

In these studies a definite amount of cystine was present after hydrolysis of the tumor extracts, and, in addition, another substance with the R_F of α-ε-diaminopimelic acid or lanthionine.

A number of reports have indicated that there are differences in the sulfur amino acid patterns of normal and malignant tissue. For example, Greenstein (10) reported that the cystine and cysteine contents of rat hepatoma were lower than in normal rat liver, but that the cystine: cysteine ratios in the two tissues were similar. Later Schweigert et al. (27) showed the proteins from various fractions of liver tumor contained less methionine and more cystine than the proteins in the corresponding fractions of normal liver. Differences in the glutathione contents of normal and malignant tissues have also frequently been reported (34). Thus in view of the chromatographic suggestion that lanthionine may be present, especially in the tumor extracts, further investigation is indicated.

The alternative suggestion of the presence of α-ε-diaminopimelic acid, which was also mentioned in these studies, bears further investigation. Its presence in malignant tissue would not be inconsistent with the following fact recently found. When a culture of an organism shown to be acid-fast in its staining properties and similar to those reported for mouse neoplasms (9) was isolated by Dr. I. Diller from the rat sarcoma No. 6 used in these studies, and was hydrolyzed in the same manner as the extracts, the hydrolysate showed a very prominent chromatographic spot with the same low R_F as the one found in the tumor extracts and mentioned above. Moreover, the hydrolysate of this culture induced heavy growth on agar plates seeded with the 173-25 coli mutant strain.

The finding that both cystathionine and lanthionine but neither

[8] This was confirmed in a personal communication.

homocystine nor cystine were found to induce growth in the coli mutant 173-25, which is dependent upon α-ε-diaminopimelic acid, is noteworthy in view of the puzzling results with the tumor extracts in connection with this mutant.

While these studies do not prove conclusively the presence in malignant tissue of an unusual substance, they suggest such a possibility. They do show striking differences in the amino acid composition between the tumor and normal tissue extracts, which are emphasized after hydrolysis. These differences supplement the findings of an earlier study (28), in which the ash, inorganic phosphorus, lactic acid, glucose, RNA and DNA also were shown to differ greatly in the normal and neoplastic tissues. The fact that a highly selective fraction of the whole tissue was here used, may explain why such differences appear to be somewhat more striking in these studies than in many previous reports on comparisons between normal and malignant tissue. However, such differences probably would not exist if there were not significant differences in the original tissue from which the extracts were made.

Summary

Amino acid analyses by the starch column technic of Moore and Stein and by paper chromatograms in phenol and butanol-acetic acid were made on unhydrolyzed and hydrolyzed alcoholic extracts of rat sarcomas and compared with extracts made in the same manner from the skeletal tissue of inbred normal, tumor-bearing and tumor-resistant rats.

A large percentage of free amino acids existed in all types of extracts. This was confirmed by analyses for the free amino nitrogen, which was 56 to 59 per cent of the total net nitrogen in the case of the tumor tissue extracts and about 18 to 23 per cent in the normal tissue extracts. A small amount of the amino nitrogen, therefore, existed in the form of peptide or whole protein.

In the unhydrolyzed extracts more glutamic and aspartic acids and proline, but less taurine, glycine and arginine, were found in the tumor than in the normal extracts.

In both cases more amino acids were released on hydrolysis, but different ones in the case of the two types of extracts. For example, in the tumor extracts more glycine, leucine and cystine appeared together with a small amount of a substance with an R_F value similar

to that of α-ε-diaminopimelic acid or lanthionine, while in the normal tissue extracts glycine also increased, and histidine, and an unidentified substance, possibly β-alanine, appeared.

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THE INFLUENCE OF ONE TRANSPLANTED NEOPLASM UPON ANOTHER IN THE SAME ANIMAL[1]

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The Technical Assistance of Nellie Harris

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There has been considerable discussion in the past concerning the influence of one neoplasm upon another and of the effect of an invasion of a tumor or its host by some virus or organism. The recent investigation of Bruwer, Donald, Higgins, McDonald and Leddy (1) concerning the inhibition of carcinoma by lymphoma, and that of Law and Dunn (6) regarding the effect of a virus on transplantable lymphoid leukemia in mice has given the subject a new impetus.

In view of the availability for experimental purposes of the Ehrlich mouse ascites tumor, a malignant rat lymphoma, several types of rat sarcomas and also two inbred strains of rats, it seemed of interest to study the influence of certain of these neoplasms upon others growing in the same animal; for instance:

1. The influence of an alien strain neoplasm, such as the mouse ascites tumor on the growth of a native tumor in an inbred rat.
2. The effect of a native lymphoma growing in an inbred rat bearing a native sarcoma.
3. The influence of a native sarcoma implanted in an inbred rat upon the growth of an alien lymphoma implanted in the same rat.

MATERIAL

Rats of the P. A. (Aptekman) and of the Lewis inbred strains were utilized in these experiments. Ten neoplasms were studied, namely, sarcomas Nos. 6, 2, 3, 4, 7, 9, 10 and 12, lymphoma No. 8 and mouse ascites tumor. The P. A. rats are a strictly inbred line of the

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King A inbred strain and the Lewis rats are an inbred strain of the Wistar Institute's pedigreed Wistar strain.

The malignant lymphoma No. 8 (7) arose spontaneously in a rat of the Lewis inbred strain, and the sarcomas[2] with the exception of sarcoma No. 6 were induced in rats of this strain. Sarcoma No. 6 was induced in a rat of the inbred P. A. strain. The lymphoma No. 8 and all of the sarcomas proved to be 100 per cent transplantable in rats of the strain in which they originated; no regressions occurred. The mouse ascites tumor grew in 100 per cent of the mice of the Swiss strain[3] in which it was transplanted.

Method

This study involved three separate experiments. 1. The influence of mouse ascites tumor injected intraperitoneally together with or followed by a dilute suspension of minced sarcoma tissue. 2. The effect of the growth of lymphoma No. 8 implanted in the left side of a rat of the Lewis strain upon the growth of a native sarcoma implanted on the right side. Five different Lewis tumors, namely, Nos. 2, 4, 9, 10 and 12 were utilized in this study. Microscopical preparations of the resulting sarcomas showed that the lymphoma cells became incorporated into the growing sarcomas. This led to a study of the transplantation of the mixed tumor through five successive passages in normal rats of the Lewis strain to determine whether the contamination was permanent. 3. The action of the alien strain lymphoma No. 8 implanted on the left side of rats of the P. A. strain bearing the native tumor No. 6 on the right side. In a few additional experiments, tissue from the alien lymphoma No. 8 and from the native sarcoma No. 6 were minced together and the mixed tumor tissue implanted into normal rats of the P. A. strain. Attempts were made to transplant the resulting sarcoma through one or more passages in rats of the P. A. strain, the purpose being to determine whether the lymphoma could be conditioned to the alien environment.

Microscopical preparations of the various tumors and differentially stained blood spreads were studied. In addition, two milliliters of blood drawn from the heart of each of the tumor-bearing rats were injected into normal rats to determine whether the neoplastic cells were present in the blood of the host.

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[2] The term sarcomas includes fibrosarcoma.
[3] Obtained from J. E. Stocker, Ramsey, N. J.

Results

Experiment 1. Influence of mouse ascites tumor in inbred rats on the growth of native sarcoma.

The results showed that one milliliter of a freshly drawn suspension of Ehrlich mouse ascites tumor injected into the peritoneal cavity of adult rats of the Lewis, the P. A. or the Sprague-Dawley strain grew vigorously, increasing in volume from the 1 ml. injected to 30 to 50 ml. within 3 to 4 days. At this time, the suspension of ascitic cells drawn from the rat was thick and milky. Shortly afterwards, however, it became amber colored and thin, containing relatively few cells; regression took place and the treated rats returned to their normal appearance. When the resistance of the treated rats was challenged by subsequent intraperitoneal or subcutaneous injections of mouse ascites tumor, the ascitic cells failed to multiply indicating that the treated rats had developed resistance to further growth of mouse ascites tumor.

The 60 rats rendered resistant to mouse ascites tumor were later implanted with sarcoma tissue, 24 with sarcoma No. 3, 22 with sarcoma No. 7 and 14 with sarcoma No. 10. None of the 60 rats that were resistant to mouse ascites tumor proved to be resistant to growth of sarcoma tissue.

Suspensions of minced sarcoma Nos. 3, 7, and 10 injected into the peritoneal cavity of normal rats of the Lewis strain grew in every one of them indicating that conditions in the abdominal cavity of the normal rats were favorable for growth of these sarcomas.

As is shown in Table 1, the influence of the growth and regression of the mouse ascites tumor in a rat upon the growth of native sarcoma tissue implanted together with or following the ascites tumor was to some extent dependent upon the particular sarcoma used. The growth of sarcoma No. 7 was inhibited in 38 per cent of the treated rats; the growth of sarcoma No. 3 was affected to a slightly lesser extent, while that of sarcoma No. 10 was not influenced by the abnormal environment. The time of injection of sarcoma cells, whether together with the ascites tumor or after it has grown for three days did not influence the results significantly.

The results obtained in experiment 1 seem to indicate that certain sarcoma cells genetically compatible with the host become adversely

TABLE 1
Resistance of Rats Injected with Ascites Tumor Alone or Accompanied by Minced Tumor Tissue

Number rats injected	Material injected	Tissue injected later	No. rats tumor regress.	Challenged with	Number tumors grew	Number rats resist.
60	ascites		60	ascites	0	60*
40	as + No. 3		12	sar No. 3	1	11
30	as + No. 7		12	sar No. 7	2	10
22	as + No. 10		0			0
20	ascites	sar No. 3	10	sar No. 3	2	8
18	ascites	sar No. 7	6	sar No. 7	1	5
12	ascites	sar No. 10	0			0
18	sar No. 3		0			0
16	sar No. 7		0			0
20	sar No. 10		0			0

Number rats*	Resist. to	Chall. with	Number tumors	No. rats resist.
24	ascites	No. 3	24	0
22	ascites	No. 7	22	0
14	ascites	No. 10	14	0

* See page 169.

influenced by changed conditions at the site of implantation, while other sarcoma cells with less exacting requirements are not affected.

Experiment 2. Influence of native sarcoma Nos. 2, 4, 9, 10 and 12 on native lymphoma growing in rats of the Lewis inbred strain.

The transplantations through five successive passages in the rats used in experiment 2 are shown in Figure 1. This experiment was repeated once increasing the number of rats used for each tumor from 46 to 92 or a total of 460 rats for the study of the five sarcomas.

In previous studies (8), it was found that lymphoma cells were present in the blood stream of the host shortly after subcutaneous implantation of lymphoma No. 8. The lymphomatous cells present in the circulatory system did not multiply to form metastatic growth in the lungs, liver or placenta of the host; nor did they form metastases in sarcoma No. 2, 4, 9, 10 or 12 transplanted into rats bearing lymphoma No. 8. The stained preparations revealed the presence of lymphoma cells within but not outside of the blood vessels of the sarcomas. Further transplantation of the sarcomas, however, resulted in the growth of a mixed neoplasm. The lymphomatous nature of the transplanted sarcomas appeared to be due to the growth of scattered

lymphoma cells released from the blood vessels during implantation of the tumor tissue. In other experiments, it was found that subcutaneous or intraperitoneal injections of as little as one-fifth of one drop of whole blood from a rat bearing lymphoma No. 8 gave rise to a lymphoma.

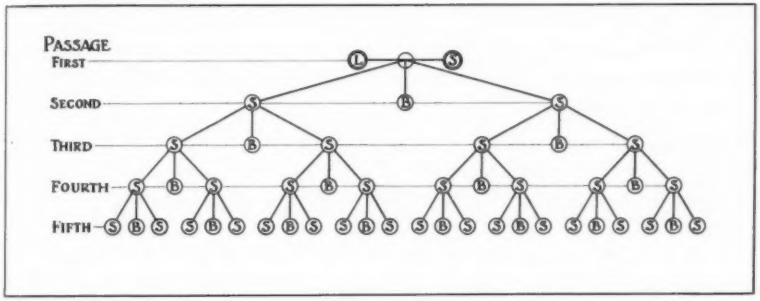

FIGURE 1

Transplantation through five successive passages of the sarcoma that grew in a rat implanted with a sarcoma on one side and lymphoma No. 8 on the other. In each passage the rat bearing the sarcoma was bled and 2 ml. of whole blood injected into a normal rat. After this, the sarcoma was implanted into two normal rats.

The results of the transplantation of the various sarcomas through five successive passages in normal rats, indicated that the presence of the lymphoma cells neither accelerated nor inhibited the growth of sarcoma cells; stained preparations exhibited the amount of sarcoma tissue characteristic of each type of sarcoma. On the other hand, the particular sarcoma used influenced the amount of lymphoma tissue present in the mixed tumor, especially in those of the later passages. Sarcomas Nos. 2 and 9 contained more lymphoma than sarcoma tissue; No. 10 contained about the same amount of each tissue; while the amount of lymphoma present in sarcoma No. 4 was less than that in the other tumors.

Results obtained in experiment 2 indicate that, owing to the presence of the lymphomatous cells in the blood of rats bearing lymphoma No. 8, the implantation at different sites of two native neoplasms, one No. 8 and the other a sarcoma, corresponded to the implantation of the two neoplasms together at the same site. Conditions in some sarcomas proved more favorable for growth of lymphoma No. 8 than those in other sarcomas.

Experiment 3. Influence of the growth of native sarcoma No. 6 on the growth of the alien strain lymphoma No. 8 implanted in rats of the P. A. strain.

In Experiment 3 the native sarcoma No. 6 implanted on the right side of the rats of the P. A. strain grew, while the lymphoma No. 8 failed to grow. Five normal P. A. rats the five normal rats of the Lewis strain, injected with blood from the rat implanted with the two neoplasms, failed to develop lymphoma. These results indicated that lymphoma No. 8 was not transplantable in rats of the P. A. strain.

When, however, the lymphoma No. 8 and the sarcoma No. 6 were cut up together and the mixture implanted into rats of the P. A. strain, the microscopical preparations of the resulting mixed tumors exhibited a considerable number of lymphomatous cells. A few of the normal rats of the Lewis strain injected with blood from the rats bearing the mixed tumor developed lymphoma while the normal rats of the P. A. strain injected with samples of the same blood failed to develop lymphoma. When the mixed sarcoma and lymphoma tumors were transplanted into other P. A. rats, the implanted rats all developed tumors, but these tumors no longer contained lymphoma cells as was shown by stained blood spreads and microscopical sections of the tumors. Rats injected with blood from rats bearing these tumors failed to develop lymphoma.

The results obtained in experiment 3 indicate that while conditions present within the growing sarcoma No. 6 were somewhat more favorable for the growth of the alien lymphoma No. 8 than those in the subcutaneous tissue of the host, nevertheless, this influence was soon lost, having disappeared by the second transplantation of the mixed tumor.

Discussion

In studies (unpublished) it was found that the peritoneal fluid of normal rats and the supernatant fluid of centrifugalized ascites tumor had a lower protein content than blood serum. Warburg and Heipler (12) showed that well developed ascites is poor in oxygen and glucose. Determinations made by Klein and Klein (5) indicated a large decrease in glucose content of the peritoneal fluid within two days after inoculation of Ehrlich mouse ascites tumor. Seibert et al. (11) found that glucose was much less in tumor extracts (0.8% to 1.8%) except that of sarcoma No. 10 (3%) than in muscle extracts (3.6 to

0.1%). Klein suggests that cells able to survive in a depleted medium such as the ascites tumor may have less exacting nutritional requirements than those that fail to survive.

Differences in nutritional requirements may explain the ability of cells of sarcoma No. 10 suspended in the mouse ascites tumor introduced into the peritoneal fluid of rats of the Lewis strain to survive and multiply during the growth and regression of the mouse ascites tumor, while sarcoma No. 3 and 7 also genetically compatible with rats of the Lewis strain were adversely influenced by this environment. Differences in requirements of this nature may also explain to some extent the more rapid multiplication of the cells of lymphoma No. 8 when incorporated into sarcoma No. 2 and 9 than when growing together with the tissue of sarcoma No. 4. On the other hand, this difference may be due to the more compact structure and less abundant circulation present in sarcoma No. 4.

It was found that sarcomas growing in rats implanted with lymphoma No. 8 became contaminated by lymphoma cells present in the blood stream of the host. For this reason, the transplantations described in experiment 2 may be said to correspond to implantations of a sarcoma and a lymphoma at the same site rather than at different sites in the same animal.

The results obtained in the studies of Bruwer et al. (1) in which suspensions of lymphosarcoma cells, Murphy-Sturm, and carcinoma cells, Walker No. 256, were implanted separately and together into rats of the non-inbred Sprague-Dawley strain are difficult to compare with the results obtained in experiments 2 and 3 owing to the genetic differences existing between the carcinoma and the lymphosarcoma and also between each neoplasm and the host animal. Nevertheless, the results of these investigators agree in general with those obtained in the present study, namely, that two tumors, one a lymphoid tumor, implanted separately into different sites in the same animal have little effect on each other, while two tumors implanted together in the same site in the same animal may influence the rate of multiplication exhibited by the neoplastic cells at different times.

The tumors that grew in experiments 2 and 3 following injections of blood from tumor-bearing rats were all lymphomas. Microscopical preparations of the various tumors did not disclose any sarcomatous tissue. This agrees with the results of earlier studies (8) in which it was found that injections into susceptible rats of 5 to 7 ml. of blood

drawn from the heart of rats bearing large sarcoma failed to give rise to tumors.

The genetic compatibility of neoplasms with the host animal (4) has been briefly stated as follows: Grafts of sarcoma that originated in inbred rats are 100 per cent transplantable in rats of the same strain, in rats of the F_1 cross between inbred rats and rats of another strain and in the F_1 backcross to the inbred strain, but not in rats of other strains.

In studies (10) of the influence of one mouse tumor upon another growing in the same animal, it was found that 1. Mice in which tumors from alien strain mice grew and regressed, became resistant to the growth of subsequent grafts of the alien strain tumor, but not to a tumor from an individual of their own strain. 2. The growth and regression of a teratoma resulting from implantation of minced embryo skin did not affect the growth of a native tumor implanted in the same mouse. 3. Mice bearing spontaneous mammary gland carcinoma were not resistant to the development of another mammary gland carcinoma. 4. When a carcinoma was implanted on one side and a sarcoma on the other side of a mouse of the same strain as the tumors, each tumor grew progressively; when, however, the host was of a different strain the tumors regressed. 5. Mice in which a primary induced tumor was developing were not refractory to the growth of grafts of induced tumors developing in other mice of the same strain.

The relationship of the host animal and the neoplasms exerts a significant influence on the results achieved, as was demonstrated recently in studies of the tumor-inhibitory activity of triethylene melamine. When triethylene melamine was fed to or injected into mice and rats of inbred strains bearing native tumors, a marked retardation of tumor growth occurred (9); when non-inbred rats bearing mongrel tumors were treated, a high percentage of cures was effected (2). However, when the treatment was applied to human beings bearing spontaneous neoplasms, quiescence of tumor growth and clinical amelioration was achieved, but so far no cures have occurred (3).

Summary

The results obtained in the present study of the influence of one transplanted tumor upon another growing in the same animal, indicate that the growth of lymphoma No. 8 and of sarcomas Nos. 2, 3, 4, 6, 7,

9, 10 and 12 in rats of the same inbred strain as that in which the neoplasm originated is not readily influenced by the presence in the host of other neoplasms either those of the same or alien strains.

Cells of certain rat sarcomas, Nos. 3 and 7, genetically compatible with the host became adversely influenced by the presence of mouse ascites at the site of implantation, while other sarcoma cells, No. 10, were not affected.

Lymphoma cells appeared in the blood stream of the host shortly after subcutaneous implantation of lymphoma No. 8, and in this way became present in sarcoma growing at another site in the same animal. Transplantation into normal rats of the Lewis strain of sarcomas thus contaminated with lymphoma cells, resulted in tumors which were not encapsulated and which were composed partly of lymphoma tissue and partly of sarcoma tissue. It was also found that injections of a few blood cells from a rat bearing lymphoma No. 8 gave rise to a lymphoma when injected into susceptible rats. The mixed nature of the sarcomas persisted through five successive passages in normal rats. Certain sarcomas, however, proved to be more favorable for growth of the lymphoma than others, depending upon the size and abundance of blood vessels in the original sarcoma.

When lymphoma No. 8, native in rats of the Lewis inbred strain, and sarcoma No. 6, native in rats of the inbred P. A. strain, were implanted at different sites in rats of the P. A. strain, the alien lymphoma No. 8 failed to grow, while the native sarcoma No. 6 grew in every one of the implanted rats. When, however, the two neoplasms were implanted together in the same rat, the conditions in the rapidly growing native sarcoma No. 6 were found to be somewhat more favorable for the growth of the alien lymphoma than those in the subcutaneous tissue of the P. A. rat.

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THE ACTION OF 1, 2, 5, 6-DIBENZANTHRACENE-9:10-ENDOSUCCINATE (SODIUM SALT) ON GROWTH OF SOME MICRO-ORGANISMS

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Investigations of the action of carcinogenic substances on micro-organisms have resulted in some interesting observations. Goldstein (1937) observed that 1,2,5,6-dibenzanthracene and 20-methylcholanthrene accelerated reproduction of *Escherichia coli communior* whereas phenanthrene did not. Cook, Hart and Joly (1938) found that an increase of yeast proliferation of about 50% was obtained with 1,2,5,6-dibenzanthracene (9×10^{-4}M) while anthracene under similar conditions had no effect. Dodge and Dodge (1937) observed that 20-methylcholanthrene profoundly affected the growth of yeasts with the production of giant cells and increased cellular differentiation. Wright and Anderson (1938) observed that water soluble oxidation products of 1,2,5,6-dibenzanthracene increased the weight of the mycelium of *Fusarium lini*.

The carcinogenic substances used by the previous authors, however, were only slightly water-soluble so that the actual concentration used was uncertain. It was felt that investigations of the action of known concentrations of a water soluble carcinogenic compound on micro-organisms might produce some interesting results.

MATERIAL AND METHODS

Water-soluble 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt),* which was shown by Parsons (6) to be carcinogenic in mice, was obtained, and its action against the following organisms was

* The sample tested in the first experiments was kindly supplied by Dr. Dorothy L. Parsons of London. Later experiments were performed with commercially obtained substances.

investigated: *Candida albicans* isolated from a case of bronchial asthma in Paris in 1947; *Torulopsis utilis* from the American Type Culture Collection; *B. mesentericus, B. subtilis, E. coli,* and *Saccharomyces cerevisiae* from the stock cultures maintained at the Biological Laboratories of Brown University.

The bacteria were grown regularly on 1% peptone broth. Yeasts were grown on a 2% glucose, 1% peptone broth, and on Hinshelwood's synthetic medium (on which bacteria also were occasionally cultivated) of the following composition:

$(NH_4)_2SO_4$	0.96 grams
K_2HOP_4	1.73 grams
KH_2PO_4	1.73 grams
$MgSO_4$	0.04 grams
NaCl	2.88 grams
Glucose	2.00 grams
H_2O (distilled)	1,000.00 ml.

All cultures were grown in 10 x 150 mm. test tubes containing 5.5 ml. of medium and 0.5 ml. of various concentrations of the sterile (autoclaved) carcinogenic substance. Control cultures were maintained for each concentration of the material studied. Bacterial growth studies were made on cultures inoculated with 0.1 ml. of a dilution made by adding 0.1 ml. of a 24-hr. culture to 5 ml. of sterile broth. Cultures for yeast growth studies were inoculated with 0.1 ml. of thoroughly mixed suspension of a young culture. All tubes were shaken before inoculation; the bacterial cultures were incubated at 37°C., the yeast cultures at 24°C. Growth was determined by readings on the Klett-Summerson Photocolorimeter, using the blue filter. Readings for bacterial growth were taken every hour and for yeast growth every two hours. The average readings for two tubes of controls and for two tubes of each concentration of test substance were plotted.

Results

In a preliminary experiment, the growth of *Candida albicans* and *Torulopsis utilis* was found to be inhibited in glucose peptone broth by 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) in a concentration of 1.4×10^{-3}M. *Candida albicans,* however, was inhibited to a greater degree than *Torulopsis utilis.*

By increasing the concentration of carcinogenic substance to

2.1 x 10^{-3}M the growth of *Candida albicans* was inhibited to greater extent than in the previous experiment while the growth of *Saccharomyces cerevisiae* was not greatly affected. Since the solutions at these concentrations (1.4 x 10^{-3}M and 2.1 x 10^{-3}M) were slightly opaque, it was thought that the carcinogenic substance may not have been completely dissolved and that the concentrations used were not expressed accurately. The carcinogenic substance, therefore, was dissolved in 70% ethyl alcohol and a 2.1 x 10^{-3}M concentration was added to the medium which gave a final 12% alcohol concentration in the medium.

TABLE 1

The difference of the growth of yeasts in the presence or absence of alcohol. M—medium containing 1% peptone and 2% glucose, ETOH—ethyl alcohol in 12% concentration, Ca—carcinogenic substance. The values shown are readings on the Klett-Summerson Photocolorimeter.

	CANDIDA ALBICANS							SACCHAROMYCES CEREVISIAE						
TIME	9HRS	14	16	19	21	23	30	9HRS	14	16	19	21	23	30
M+ETOH	9	18	19	19	23	29	41	36	64	91	131	170	219	330
M+ETOH+CA	0	2	3	4	6	6	6	0	10	59	90	107	152	261
M	62	97	114	145	198	224	260	114	177	235	292	320	355	375
M+CA	2	4	4	4	4	4	7	104	169	180	238	259	289	330

In this table, it may be seen that the growth of *Candida albicans* and *Saccharomyces cerevisiae* in medium containing alcohol was not as great as in the medium without alcohol. Following addition of the carcinogenic substance to the medium, with and without alcohol, the growth of these two organisms was suppressed during the first hours. Later, suppression was complete for *C. albicans*, but only partial for *Saccharomyces cerevisiae*. This would indicate that the effect of the carcinogenic substance on the growth of the organisms was independent of alcohol and that solubility in aqueous solution, at the concentration used, was probably complete. In all other experiments only the aqueous solution of carcinogenic substance was used.

The difference in action of 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) on the growth of *Candida albicans* and *Saccharomyces cerevisiae* in different media was studied using 2.1 x 10^{-3}M

concentration of this substance. The results of this experiment are presented in Figure 1.

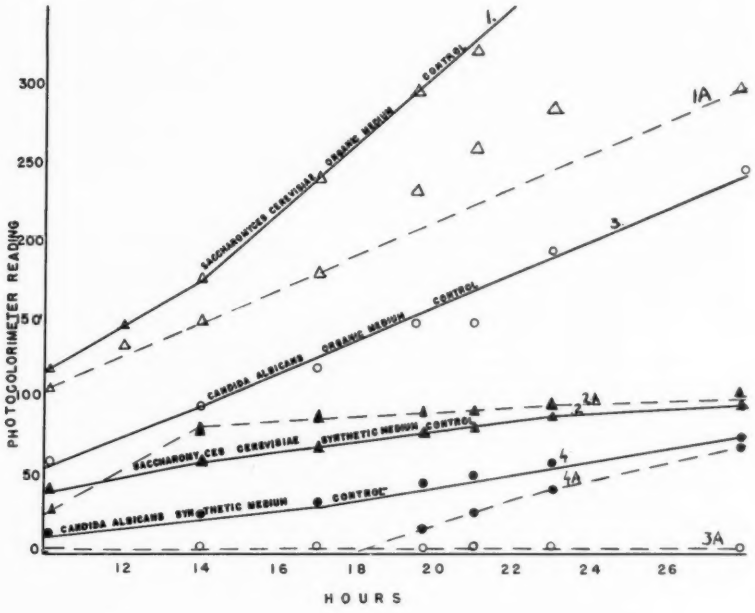

FIGURE 1

The action of water soluble carcinogenic substance on *Candida albicans* and *Saccharomyces cerevisiae* in glucose peptone and synthetic medium.

1 and 3:	*Candida albicans* and *Saccharomyces cerevisiae*, respectively, in glucose peptone broth.
1A and 3A:	*Saccharomyces cerevisiae* and *Candida albicans*, respectively, in glucose peptone broth plus carcinogenic substance in concentration 2.09 x 10^{-3}M.
2 and 4:	*S. cerevisiae* and *C. albicans*, respectively, in synthetic medium.
2A and 4A:	*S. cerevisiae* and *C. albicans*, respectively, in synthetic medium plus carcinogenic substance.

In this figure, it can be seen that growth of *Candida albicans* in glucose peptone broth was more markedly inhibited than the growth of *Saccharomyces cerevisiae*. When these two organisms were grown in the same concentration of the carcinogenic substance in synthetic medium, the growth of *Candida albicans*, although retarded for 18 hours, was not inhibited as greatly as in the glucose peptone broth.

On the other hand, the growth of *S. cerevisiae* was stimulated rather than inhibited.

At the end of this experiment, subcultures from the glucose-peptone, test and control media in which *C. albicans* was grown, were made to Sabouraud's glucose agar slants and glucose peptone broth. Growth of cultures made by transfers from the test media were retarded for 48 hours with respect to those transfers from the control medium but they fermented glucose and maltose to approximately the same extent as the controls.

The action of 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) on the growth of gram positive and gram negative bacteria was studied. *Escherichia coli* and *Bacillus mesentericus* were grown in peptone broth to which the carcinogenic substance was added in the following concentrations: 1.4 x 10^{-3}M, 1.4 x 10^{-4}M and 1.4 x 10^{-5}M. The results of this experiment are presented in Figure 2.

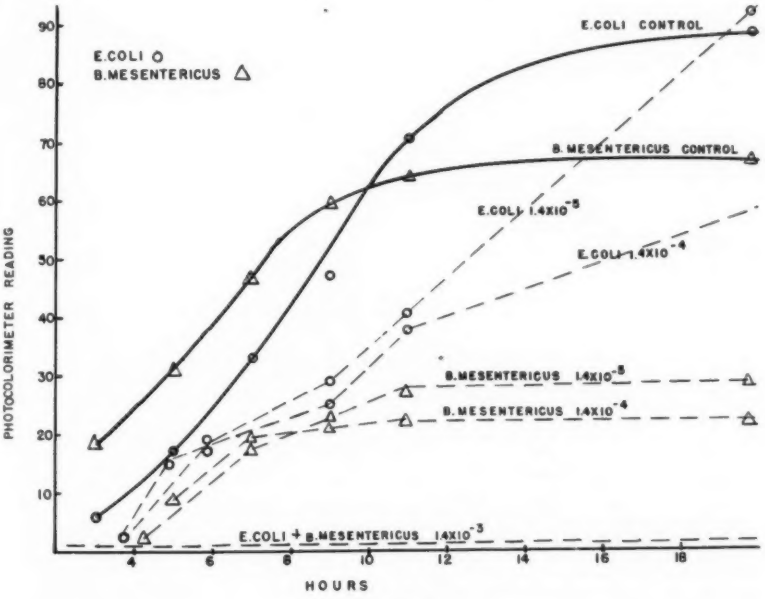

FIGURE 2

The action of different concentrations of water soluble carcinogenic substance on *B. mesentericus* and *B. coli*.

In this figure, it can be seen that a concentration of 1.4 x 10^{-3}M inhibited the growth of both organisms. *B. mesentericus* was inhibited markedly also by concentrations of 1.4 x 10^{-4}M and 1.4 x 10^{-5}M. Although a concentration of 1.4 x 10^{-4}M greatly inhibited the growth of *E. coli,* it should be noted that a concentration of 1.4 x 10^{-5}M stimulated it to the point where growth in the control tube became stationary.

Since *E. coli* showed a tendency to be stimulated to growth by a concentration of 1.4 x 10^{-5}M, the effect of higher dilutions of the carcinogenic substance on *B. mesentericus* was studied. This organism was grown in the synthetic medium for these studies because this medium was found to be less favorable for growth and a possible stimulating effect caused by an added substance would be more noticeable. The results of this study are presented in Figure 3.

It can be seen that concentrations of 1.4 x 10^{-3}M and 1.4 x 10^{-7}M

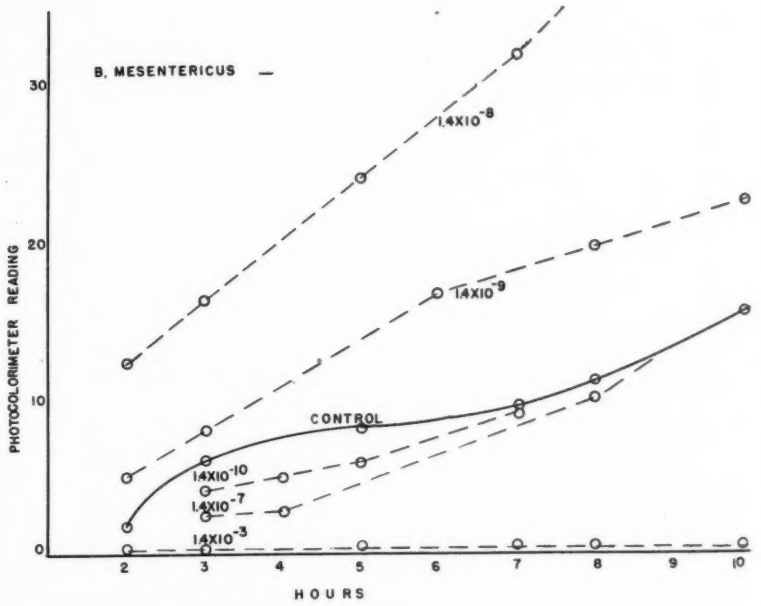

FIGURE 3

The action of different concentrations of water soluble carcinogenic substance on *B. mesentericus* in synthetic Hinshelwood medium.

inhibited the growth of *B. mesentericus* while a concentration of 1.4 x 10^{-8}M greatly stimulated it. Growth in concentrations in the range of 1.4 x 10^{-9}M and 1.4 x 10^{-10}M tend to approach that of the control.

The action of 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) on the growth of *B. subtilis* in peptone broth proved to be similar to that of *B. mesentericus* as shown in Figure 4.

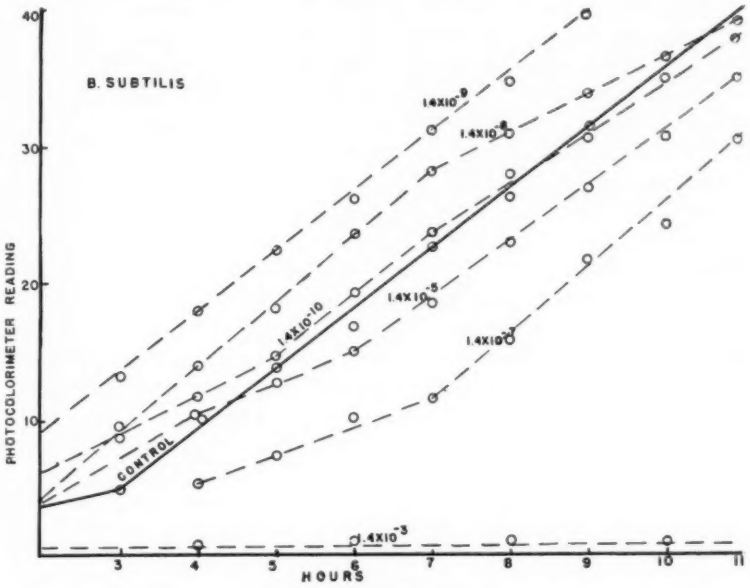

FIGURE 4
The action of different concentrations of carcinogenic substance on *B. subtilis* in glucose peptone medium.

In Figure 4 it can be seen that the growth of *B. subtilis* was inhibited by concentrations of 1.4 x 10^{-3}M and 1.4 x 10^{-7}M but that it was stimulated by a concentration of 1.4 x 10^{-9}M.

A study of the cultural characteristics, morphology, staining reactions, fermentation reactions and antigenicity of those cultures of *E. coli, B. mesentericus,* and *B. subtilis* which were used in these experiments revealed no changes as a result of having been subjected to the action of 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium

salt). Also, respiration studies of *B. mesentericus* in peptone broth with different concentrations of the carcinogenic substance and in peptone broth without the carcinogenic substance showed no differences.*

Discussion

The water soluble 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) seems to be useful for investigating the action of a carcinogenic substance on the growth of micro-organisms.

The most striking effect of this carcinogenic substance is its capacity to check the growth of micro-organisms in high concentration and occasionally to stimulate the growth of the same micro-organisms in greater dilutions. Stimulation of growth was obtained with only one sample of carcinogen, namely that of Dr. Parsons. Other samples from commercial sources did not exert a stimulatory action, although all of them inhibited growth. From this it would appear possible that a stimulating effect may not be an inherent property of this substance. These observations are in accord with what is known about the action of carcinogenic substance on animal tissues. It is known, for example, that 1,2,5,6-dibenzanthracene, in small quantities possesses known carcinogenic capacity, but the same substance in greater doses also possesses an inhibiting action on growth of animal tissue. Lee (1947) stated that "single injection of 5, 10 and 20 mg. of 1,2,5,6-dibenzanthracene produces a prolonged inhibiting effect on the growth of young rats, and the effect produced varies as the dosage." Haddow, Scott and Scott (1937) found that "1,2,5,6-dibenzanthracene produced an immediate, constant and long continued reduction in the rate of growth of young rats."

The capacity of stimulating proliferation, occasionally obtained, cannot be considered as corresponding to the development of neoplasia. There is no indication of alteration of the morphology, antigenicity and metabolic characteristics of the bacteria studied. The general distinctions between Gram positive and Gram negative bacteria were found also in this study; i.e. *B. mesentericus* was distinctly more susceptible to the stimulatory action of carcinogenic substance than was *E. coli.*

Among the yeasts studied, *Candida albicans* was more susceptible

* Respiration studies by the Warburg technique were made by Dr. G. Sherman of the Department of Biology of Brown University.

to action of the carcinogenic substance than was *Saccharomyces cerevisiae* or *Torulopsis utilis*. Also, slight changes in the morphology of *Candida albicans* encourage further study of this subject.

Summary

Water-soluble 1,2,5,6-dibenzanthracene-9:10-endosuccinate (sodium salt) is capable of checking growth of several micro-organisms and occasionally, in greater dilution, stimulates growth. Differences in action were observed with *Candida albicans, Saccharomyces cerevisiae, Torulopsis utilis, B. subtilis* and *Escherichia coli*. The inhibition of growth of *Candida albicans* was more marked than that of *Saccharomyces cerevisiae* and *Torulopsis utilis*. Also the effect on the growth of *B. mesentericus* and *B. subtilis* (Gram positive bacteria) was greater than the effect on the growth of *E. coli* (Gram negative organism).

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Growth, 1954, **18**, 187-189.

BOOK REVIEWS

Symposium on Effects of Radiation and other Deleterious Agents on Embryonic Development. Sponsored by The Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, April 20, 21, 1953. Journal of Cellular and Comparative Physiology, Vol. 43, Supplement 1, May, 1954.

This stimulating symposium brought together at Oak Ridge a group which comprised experimental and clinical radiologists, embryologists, geneticists, physiologists, cytologists and pathologists. The published volume comprises eleven papers with a report of the discussion following each one.

The two principal themes of the conference are stated in the introduction by Alexander Hollaender: first, the information concerning basic biological problems that can be gained from the use of agents that interfere with normal processes; and second, an understanding of the effects to be expected and guarded against from the use of high energy radiation for diagnostic and therapeutic purposes, as well as in nuclear weapons.

The application of deleterious agents to early developmental stages brings the discussion at once to problems of differentiation, both at the morphological and at the cellular level. The first papers deal with radiation applied to embryos of the rat (James G. Wilson), mouse (Liane Brauch Russell and W. L. Russell) and amphibia (Roberts Rugh). Starting from the finding in all cases that treatment in the earliest stages does not yield gross abnormalities, the discussions then proceed to the consideration of "critical periods" in later development, either in general sensitivity or for the production of particular effects.

A paper by Samuel P. Hicks considers effects on the developing nervous system of the rat, comparing the action of ionizing radiations with that of hormones and radiomimetic drugs. This is followed by other papers on the action of chemical agents—nitrogen mustards on amphibia by Dietrich Bodenstein, cortisone and other hormones on mouse embryos by F. Clarke Fraser, H. Kalter, B. E. Walker and T. D. Fainstat, and a group of chemicals that influence carbohydrate metabolism on chicken embryos by Walter Landauer. The effect of maternal vitamin deficiencies on mammalian offspring is discussed by Josef Warkany. The discussions center around mechanisms of action as related to cellular metabolism, to genetically controlled

syndromes and to stages of development. Background for the latter consideration is given in a paper by B. H. Willier.

A paper by James M. Dent and Ernest L. Hunt demonstrates the use of radiotracer techniques in embryological research.

The dangers arising from diagnostic irradiation of human mothers during the first few weeks of pregnancy is stressed by Rugh in the discussion of the paper by Hicks. A paper presented in an evening session by James Yamazaki, Stanley and Phyllis Wright represents a hitherto unpublished study of the offspring of ninety-eight women who were found to have been pregnant at the time of the Nagasaki explosion. Fetal death, neonatal death, infant death and mental retardation were significantly higher in the cases of thirty mothers who were closer to the center of the blast and who suffered major radiation injuries than in the cases of sixty-eight mothers who were farther away from the blast.

In a summary of the conference, Paul Weiss makes the following points: (1) that embryogenesis operates with "the whole plasma and the genetic system in it in a continuous series of transformations", "a large number of separate chains of events" that are "interrelated in increasing complexity"; (2) that "an agent may undergo a number of modifications in its course through the organism", and (3) that in considering dosage relationships one should think in terms of populations of cells with increasing numbers, where the initial action of an agent may be followed by chains of secondary reactions emanating from the injured cells. Under such conditions, no single agent or single effect can account for the whole result.

The rather large number of misprints is offset by the clarity of the charts and photographic plates. This volume should make a valuable addition to the libraries of institutions or individuals who are interested in problems of radiation or development.

ELIZABETH UFFORD GREEN

The Cultivation of Animal and Plant Cells. Philip R. White, Research Associate, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The Roland Press Co., New York, 1954. $6.00.

The "Foreword" of this valuable book, states that "this is not a book on 'tissue culture.' It deals with the cultivation of cells." "The term 'tissue culture' has always been a misnomer." One may well

take issue with the author regarding the term "tissue culture." Every culture begins with a tissue or a piece of tissue ("An aggregation of cells, fibers and various cell-products, forming a structural element." Dorland) and upon cultivation ("To bestow care and labor upon, with a view to valuable returns;" Webster), remains a tissue, that is, it consists of cells and their products. Cultivated animal tissues contain cells and cell-products. Plant tissues remain plant tissues and consist of cells, cellulose walls, and other metabolic products. Organs are tissues and organ culture, which the author includes, is tissue culture. Even a culture that begins with a single cell which has been isolated from a tissue culture, emphasizes the necessity and importance of bestowing care and labor on the products of the cell, which are as important as the cell itself.

The first chapter, "The cell versus the organism", is stimulating and thought provoking. Some of the fundamental biological problems which tissue culture technique may help to solve are presented. The second chapter is on "The history of cell culture." The 3rd chapter, on "The Living Material," suitable for cultures includes the fertilized egg, which the author states "is in certain theoretical respects the ideal subject for cell culture." The fertilized egg is an entire organism. The interior of an entire organism at any stage, morula, blastula, embryo, fetus or adult, affords the ideal environment for its tissues and cells, but the cultivation of entire organisms is neither tissue culture or cell culture.

The chapters on the Laboratory, Nutrients, Types of Cultures, Setting Up Cultures, Growth Measurements are adequately treated, with many interesting suggestions for both animal and plant cultures. The term "replications," ("an answer, a reply, a repetition, a copy." Webster), which the author and others use for similar or like cultures, is a little grandiose. The last chapter is on "Applications" of cell culture techniques, which "to date fall for the most part into six categories." They are according to the author, cellular nutrition, cellular metabolism, hormone relations, morphogenesis, pathology, and genetics. Perhaps cytology should be added. An appendix, "Some simple techniques for beginners" is also designed for class use. Even high school students can watch cells divide.

This scholarly (spiced with classical quotations and references) and useful book, in which the author has emphasized the role of cells in biology, hence its title. should have many "replications" in the hands of biologists.

WARREN H. LEWIS